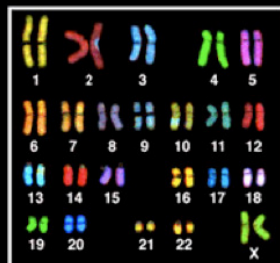
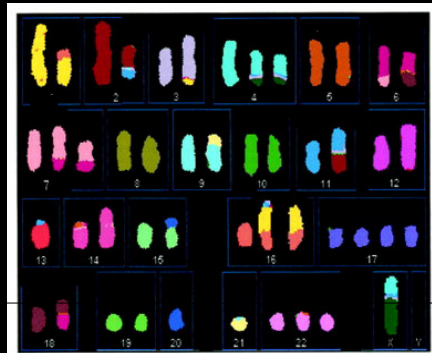




Repair of Broken Chromosomes and Maintenance of Chromosome Stability

Jim Haber

Brandeis University



Genome instability in tumor cells

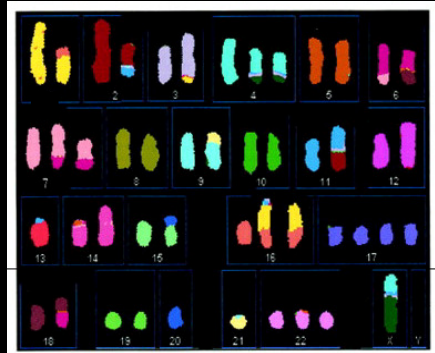
Truncations
Translocations
Inversions
Duplications
Amplifications

Deletions

Mutations

Mis-segregation

Abdel-Rahman et al. PNAS 98: 2538 (2001)



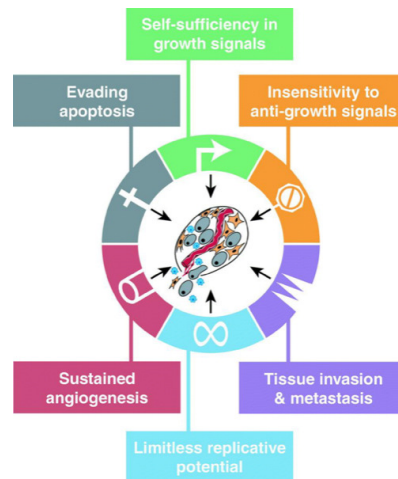
Genome instability in tumor cells

Caused by defects in the repair of chromosome breaks
or
defects in the DNA damage checkpoint

Abdel-Rahman et al. PNAS 98: 2538 (2001)

For a normal cell to become a cancer cell, it must accumulate many mutations (≥ 6) before they are transformed into cancer cells.

Stem cells or more differentiated cells undergo only a limited number of cell divisions, so how do they accumulate this many mutations?



Robert Weinberg, MIT

An Oncogene-Induced DNA Damage Model for Cancer Development

Thanos D. Halazonetis,^{1*} Vassilis G. Gorgoulis,² Jiri Bartek³

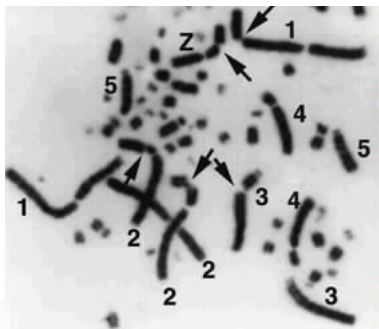
Of all types of DNA damage, DNA double-strand breaks (DSBs) pose the greatest challenge to cells. One might have, therefore, anticipated that a sizable number of DNA DSBs would be incompatible with cell proliferation. Yet recent experimental findings suggest that, in both precancerous lesions and cancers, activated oncogenes induce stalling and collapse of DNA replication forks, which in turn leads to formation of DNA DSBs. This continuous formation of DNA DSBs may contribute to the genomic instability that characterizes the vast majority of human cancers.

To which we wish to add:

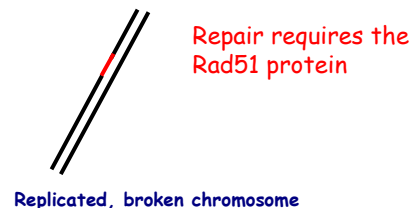
Even when DSBs are "perfectly" repaired by gene conversion, the increase in frequency of repair leads to a dramatic increase in the rate of mutagenesis. The increased rate of mutation may directly contribute to the accumulation of additional mutations in precancerous cells.

A major source of genome instability comes from broken chromosomes.

Breaks arise spontaneously because the replication process is surprisingly fragile.



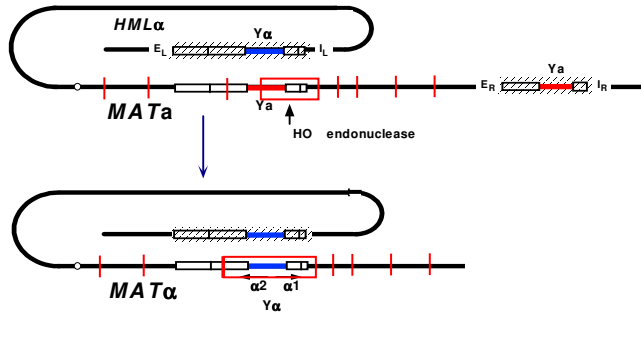
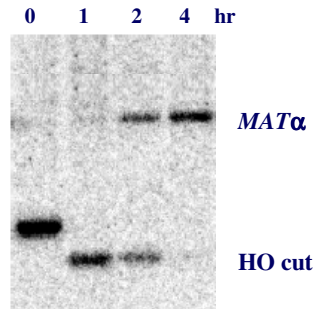
DSBs revealed in vertebrate cells after the Rad51 repair protein is depleted



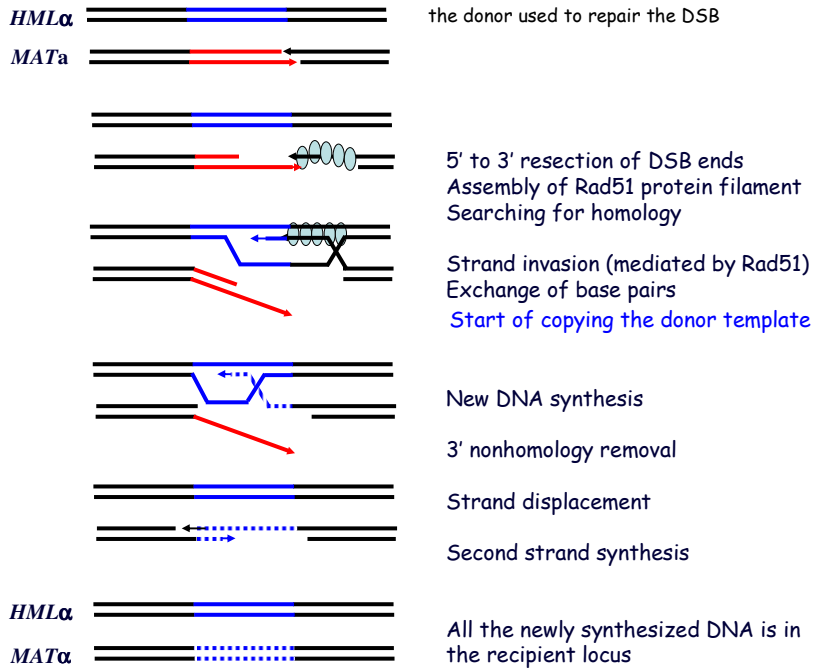
Chromosome breaks also arise from X-rays, chemical exposures

<http://www.nature.com/emboj/journal/v17/n2/abs/7590776a.html>

Physical monitoring of
MAT switching by Southern blot analysis

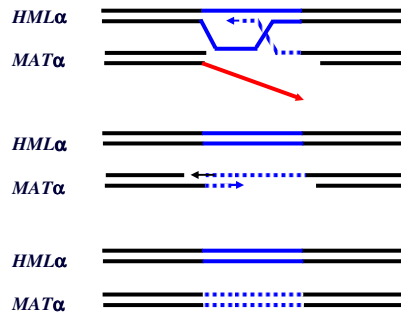


Connolly, White and Haber 1988



What is the mutation rate and the spectrum of mutations for the DNA sequences that are copied during DSB repair?

What is the role of mismatch repair? What's an "old" strand?
What are the roles of different DNA polymerases?

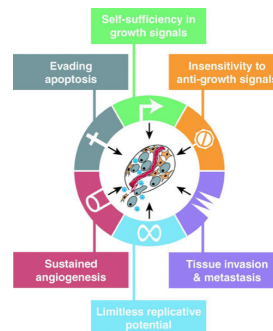


Bottom line:
The rate of mutation is 250-1000x over the basal level

Many of the mutations suggest that they arise by replication slippage.

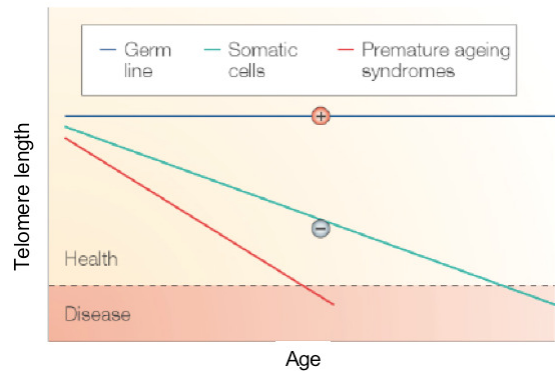
Mutations that promote chromosome breakage (and repair) will also increase the rate of mutation at those sites

This increased mutation rate may help account for how cells can accumulate so many mutations before they are transformed into cancer cells.



Cancer and cell immortality

As cells divide their chromosome ends (telomeres) get progressively shorter and cells undergo senescence



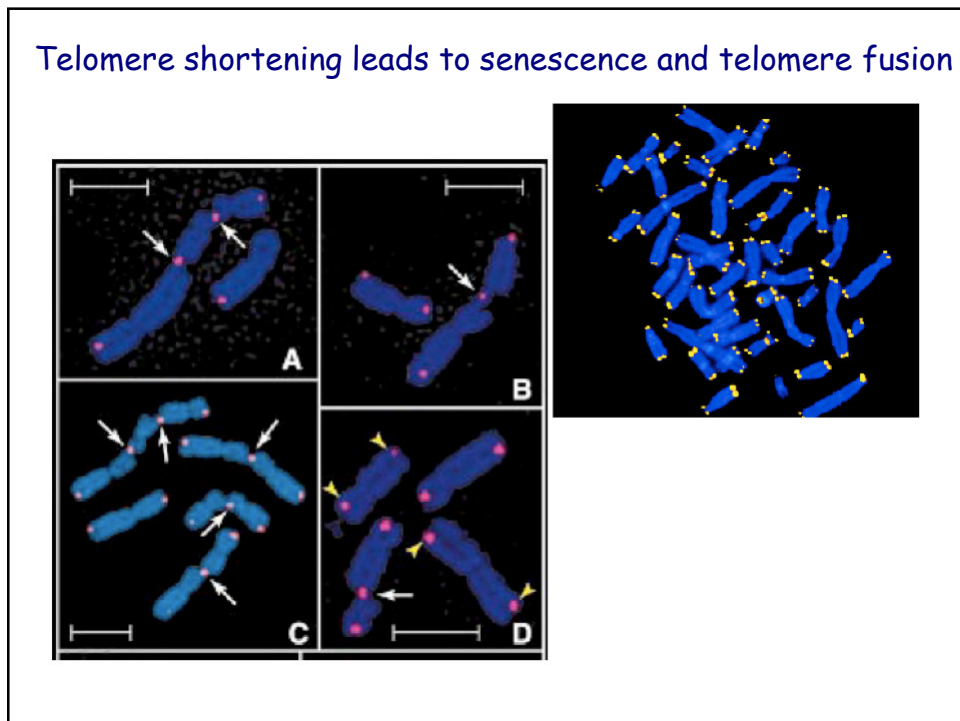
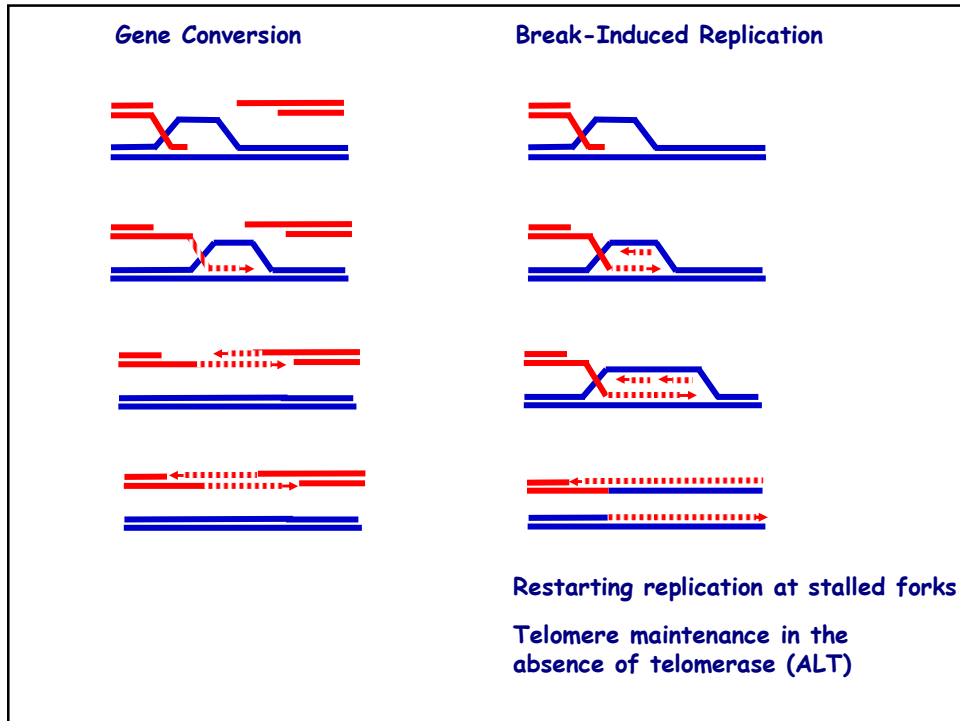
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Cancer and cell immortality

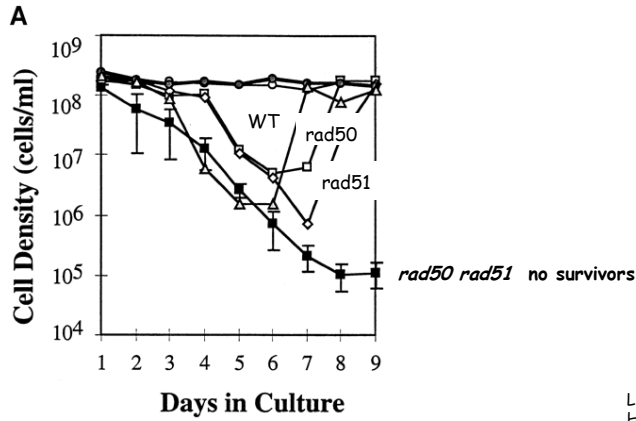
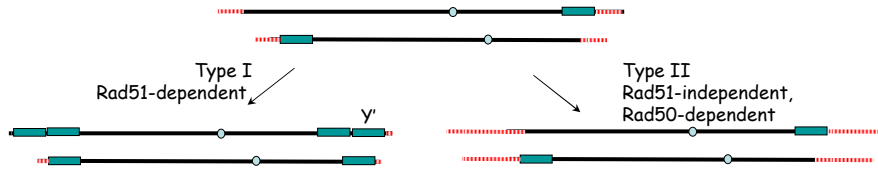
As cells divide their chromosome ends (telomeres) get progressively shorter and cells undergo senescence

Some tumor cells regain the ability to elongate telomeres by Alternative Lengthening of Telomeres (ALT)

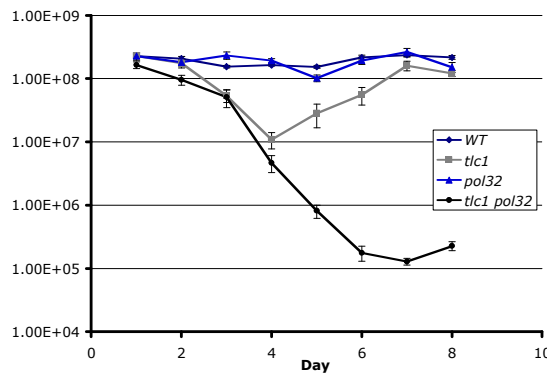
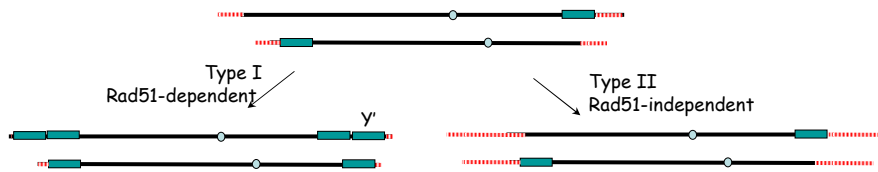
In yeast, ALT occurs by a DNA repair process call Break-Induced Replication, a process that requires the Pol32 protein



Without telomerase, yeast cells survive by recombination-based mechanisms



Without telomerase, yeast cells survive by recombination-based mechanisms



Pol32 is required for both types of telomere maintenance without telomerase, suggesting that Break-Induced Replication is required

Miyuki Yamaguchi

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